



NTP
National Toxicology Program

Research Concept: Artificial Butter Flavoring, Diacetyl and Acetoin

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NIEHS/NTP





Nomination

- 1994 Diacetyl nominated by NCI for mechanistic, metabolism and carcinogenicity testing by oral route. Based on widespread use and structure-activity.
 - Not palatable in drinking water
 - Too volatile in food
 - Metabolized almost completely to CO₂ after gavage
 - Study deferred
- 2006 Diacetyl, acetoin and artificial butter flavoring nominated by UFCW for long-term testing for respiratory and other toxicity, and carcinogenicity by inhalation route.
 - Outbreaks of obliterative bronchiolitis (OB) in workers at microwave popcorn packaging plants
 - Thousands more workers potentially exposed in food industry
 - Inhalation toxicity data are needed for exposure regulation



Rationale for Proposed Studies

- Human Health Significance
 - Workers in microwave popcorn, candy, snack food, baking and flavoring industries are exposed to butter flavoring and components
 - Exposed workers diagnosed with obliterative bronchiolitis
 - Debilitating and often progressive and fatal disease
 - Irreversible, no treatment
 - Insidious development (no over exposure incident)
 - Etiology and progression of OB are unknown - biomarkers for early detection and intervention strategies are needed
- Inhalation toxicity data for diacetyl, acetoin and artificial butter flavoring are lacking
 - Data are needed to regulate occupational exposure
 - Toxic components of butter flavoring are not known
 - Cases of OB in popcorn, flavoring, food and diacetyl production industries all involved mixed exposures



Background: Microwave Popcorn Packaging Workers

- 2000 - Eight employees diagnosed with obliterative bronchiolitis
- NIOSH health hazard evaluation
 - Strong correlation between cumulative exposure to butter flavoring and extent of airway obstruction
 - Flavoring mixers – highest exposure and highest incidence of OB
- Post-2000 – increasing number of cases of OB diagnosed in industries where butter flavoring is used or made





Properties and Uses of Artificial Butter Flavoring

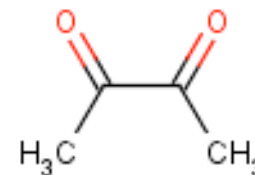
- Complex mixture – >150 VOCs detected from heated butter flavoring
- Multiple producers, proprietary formulas
- Used as a butter flavor alternative in a variety of food products
- Contains diacetyl and acetoin



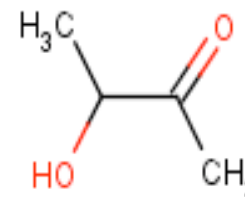
Properties and Uses of Diacetyl and Acetoin

- Major volatile components of artificial butter flavoring
- Flavoring and aroma carriers
- Naturally present in butter, beer, coffee, dairy products
- Direct-acting ketones
 - Reactive without metabolism
 - Volatile, water soluble
 - Contact irritants

Diacetyl



Acetoin

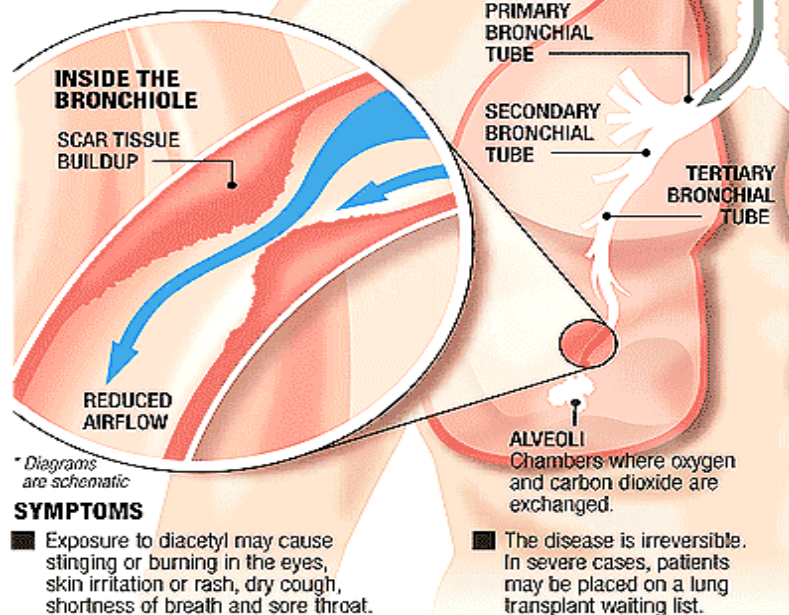




Butter Flavoring-Induced Obliterative Bronchiolitis

EXPOSURE TO DIACETYL AND ONSET OF BRONCHIOLITIS OBLITERANS

- A** Diacetyl enters the body when a person inhales vapors, droplets of spray or dust containing the chemical.
- B** The chemical causes inflammation, scarring and constriction of the bronchioles (tiny airways in the lungs). The constriction causes a loss of lung function and can be fatal.



SYMPTOMS

- Exposure to diacetyl may cause stinging or burning in the eyes, skin irritation or rash, dry cough, shortness of breath and sore throat.

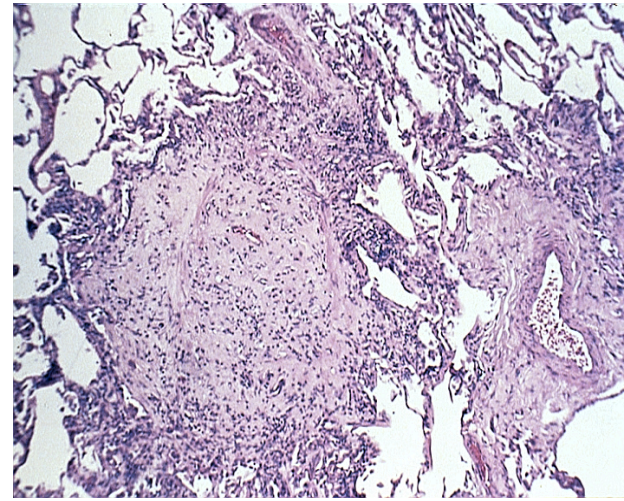
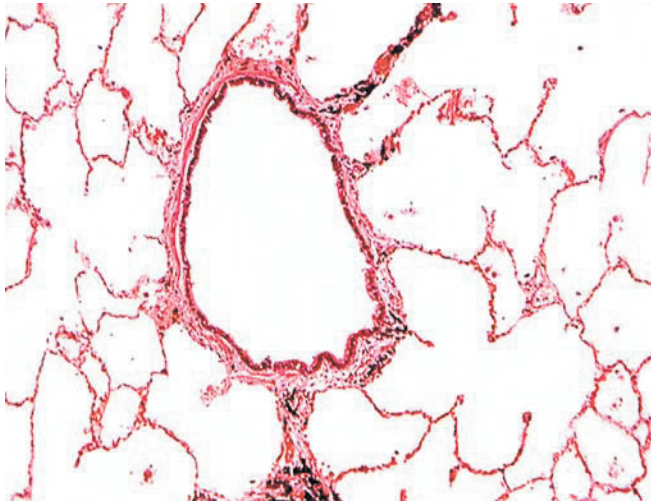
- The disease is irreversible. In severe cases, patients may be placed on a lung transplant waiting list.

Sources: Centers for Disease Control and Prevention, California Department of Health Services

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Obliterative Bronchiolitis



- Reference: http://www.som.tulane.edu/classware/pathology/medical_pathology/New_for_98/Lung_Review/Lung-32.html



Hypotheses:

- Respiratory toxicity of inhaled artificial butter flavoring is due to diacetyl
 - Acetoin and other components do not contribute to toxicity
- There is no systemic absorption and toxicity of butter flavoring or its components
 - Toxicity is limited to the site of contact
- Butter flavoring induced OB is immune-mediated
 - Diacetyl is a potent sensitizer
 - OB in lung transplant rejection – immune component



Proposed Studies of Butter Flavoring, Diacetyl and Acetoin

Aim 1: Evaluate contribution of diacetyl and acetoin to respiratory toxicity of butter flavoring

- Quantify toxicity of flavoring containing known amounts of diacetyl and acetoin
- Compare toxicity of flavoring with equal concentrations of diacetyl, acetoin, or diacetyl + acetoin

Aim 2: Evaluate systemic distribution and toxicity after inhalation of butter flavoring, diacetyl or acetoin

- Evaluate histopathology in pulmonary and extrapulmonary tissues
- Clinical chemistry for systemic toxicity
- Binding sites - radiolabeled or immunolabeled diacetyl and acetoin



Proposed Studies of Butter Flavoring, Diacetyl and Acetoin

Aim 3: Investigate immune-mediated mechanism for OB

- Immunotoxicity test battery after inhalation exposure
Antibody response, splenic subpopulations, NK cell assay, lymphoproliferative response, cytotoxic T lymphocyte assay
- Dermal sensitization + inhalation challenge

Aim 4: Obtain inhalation dose-response and NOEL data

- Subchronic and chronic inhalation studies
- Evaluate other exposure profiles, e.g. intermittent high-concentration exposures



Preliminary NTP Studies on Diacetyl

- Studies coordinated with NIOSH investigators
- Evidence for diacetyl as a toxic component of artificial butter flavoring
 - Diacetyl is a reactive contact irritant
 - Diacetyl is the predominant VOC in butter flavoring vapors
 - OB diagnosed in workers exposed to diacetyl (all cases involved simultaneous exposure to other chemicals)
- Objectives:
 - Obtain dose-response data and establish a NOEL for diacetyl in mice
 - Develop an animal model to study OB for lung transplant rejection

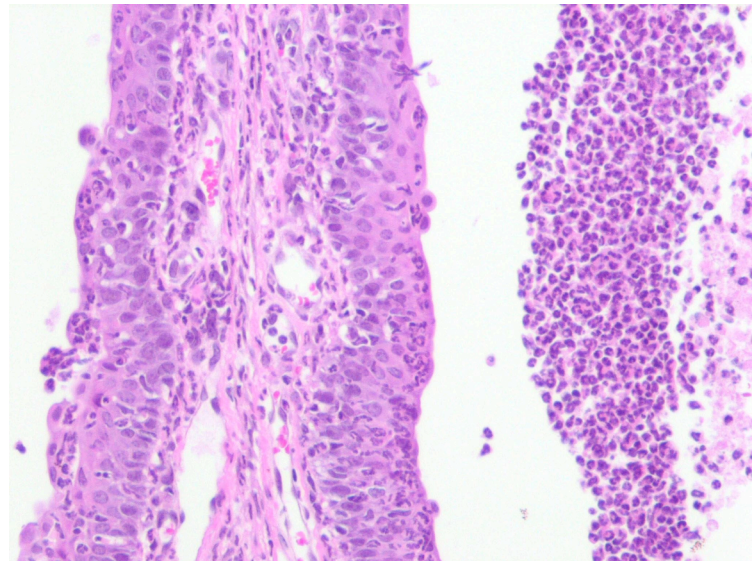


Diacetyl - subchronic inhalation

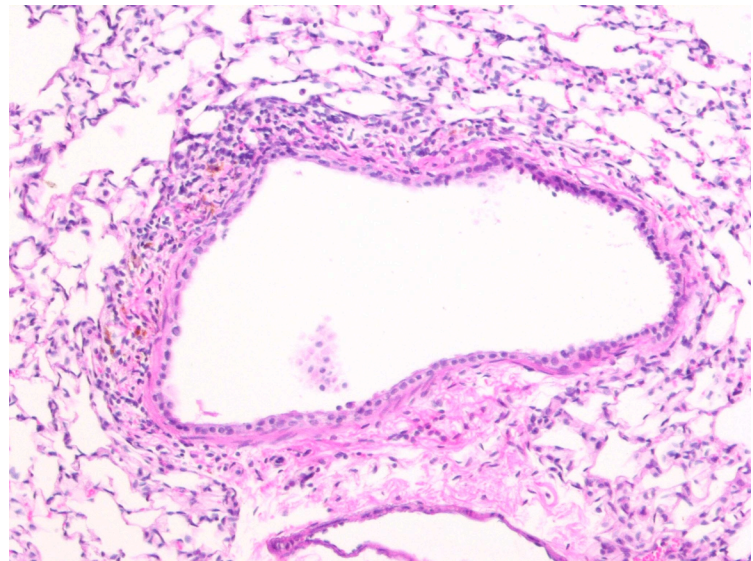
Male, C57/Bl/6 mice exposed to 0, 25, 50 or 100 ppm diacetyl vapors for 6h/d, 5d/w/12w.

Nose and URT were primary targets in mice.

A. Nasal turbinates, level I.
Suppurative exudate and squamous metaplasia of septum.



B. Small bronchial branch.
Lymphocytic bronchitis and bronchiolitis.



Precursor to OB?

Longer exposure required?



Diacetyl – oropharyngeal aspiration

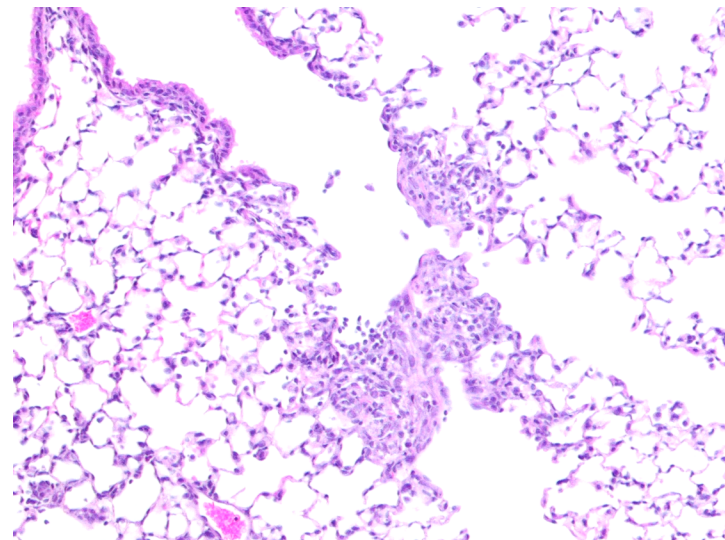
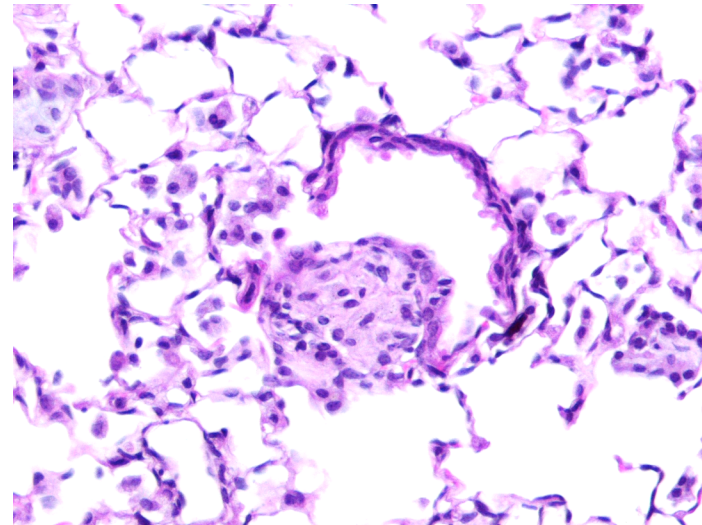
Increase dose to airways and eliminate nasal toxicity

Mice treated 1x with 200 or 400 mg/kg diacetyl by oropharyngeal aspiration and evaluated 4 days later.

- A. Fibrohistiocytic lesion protruding into terminal bronchiole.
- B. Fibrohistiocytic lesion at junction of terminal bronchiole and alveolar duct.

At sufficient dose to distal AW, diacetyl can cause lesions typical of OB

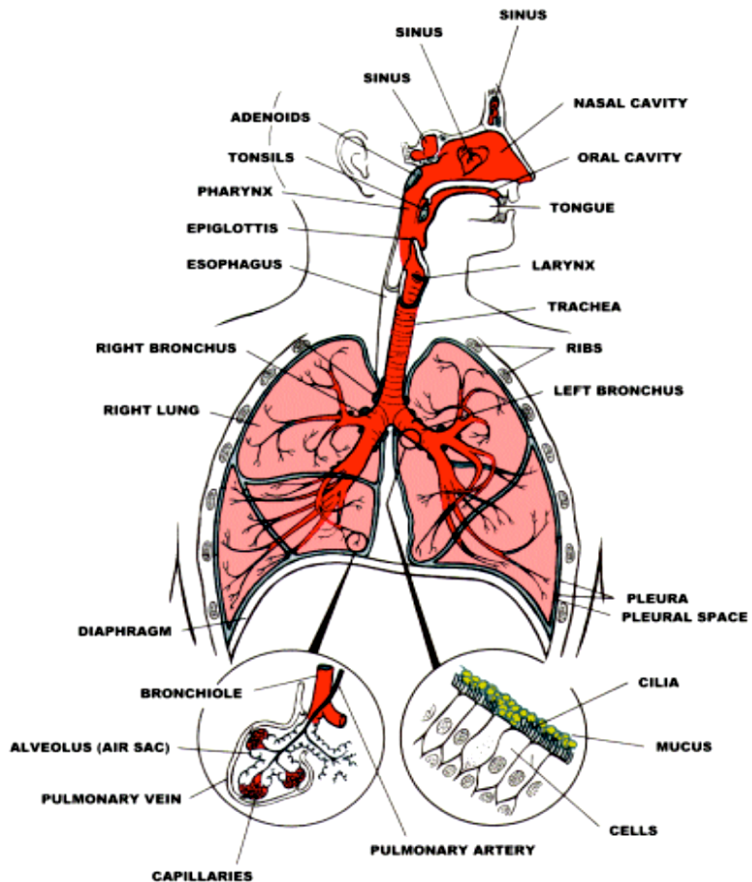
Although aspiration of diacetyl is nonphysiological, may be suitable to use for animal model of OB





Species Difference in Target Sites

- Rodents - *Toxicity – nose and URT*
 - Obligate nose breathers
 - All inhaled air scrubbed by nose
 - Complex nasal cavity anatomy
 - Concentrates chemical in URT
- Humans - *Toxicity – Bronchioles*
 - Nose and mouth breathers
 - Less inhaled air scrubbed by nose
 - Chemical reaches distal airways
 - Bronchioles – no mucous layer





Key Considerations in Study Design

- Artificial butter flavoring is a complex mixture (which mixture?)
- Potential synergistic / additive toxicity of butter flavoring components
- Anatomical and physiological species differences
- Extrapolation of inhalation toxicity data from animals to humans



Significance and Public Health Impact

- Provide inhalation toxicity data needed to regulate occupational exposures to butter flavoring, diacetyl and acetoin
- Identify toxic component(s) of artificial butter flavoring
- Provide information on the etiology and progression of flavoring induced obliterative bronchiolitis
- Potentially identify biomarkers for early detection and develop intervention strategies